

To: Office of the Secretary
Federal Communications Commission
Washington, DC 20554

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The EMR Policy Institute, Inc., a non-profit educational organization based in Marshfield, VT, submits the following comment in response to the Commission's request for comment on the extent of any effect of communications towers on migratory birds and whether any such effect warrants regulations specifically designed to protect migratory birds (pars. 32 et seq.).

I. Disorientation of Migratory Birds

There is a major omission from the Commission's focus on causes for "blind collisions" with communications towers – the effects of radiofrequency transmissions (as distinguished from lighting) disorienting migratory birds and affecting their natural navigation ability. Attached are two scientific studies addressing this potential cause of towerkills. These studies merit the adoption of precautionary Commission actions described below (in Section III) and also warrant further scientific inquiry and study by the Commission.

II. Destruction of Bird Habitats

There are two important recent studies (also attached) establishing that communications towers operating at low intensities in the radiofrequency range have harmful effects on

nesting behaviors and habitats in the vicinity of towers. The white stork study from Spain demonstrates the effects of cell tower emissions in causing destructive bird behavior and reducing reproduction of offspring on nesting birds within 200 meters of cell towers. The mouse infertility study from Greece demonstrates the destructive effects on the natural food chain in bird habitats within 448 meters of broadcast towers. These studies also support the need for immediate precautionary Commission actions pending more comprehensive independent scientific inquiry and studies by the Commission.

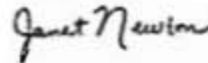
III. Precautionary Commission Actions

In view of the present high mortality rates for migratory birds at tower sites, The EMR Policy Institute recommends that the Commission take the following immediate precautionary actions pending completion of more comprehensive and definitive independent studies of the effects of radiofrequency radiation from communications towers in causing migratory bird infertility or deaths.

- A. Prohibit the nighttime operation of communications towers within 5 miles of any known migratory bird flyway.
- B. Prohibit the siting or operation of any communications towers or antennas within 2 miles of sensitive bird nesting areas or habitats of endangered and/or listed species.

Respectfully submitted,

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communities, values of b_{ij} were selected from uniform distributions between 0 and -0.1 (plant–plant competition), -0.3 (herbivores \rightarrow plants; that is, the effect of herbivores on plants), 0.1 (plants \rightarrow herbivores), -0.1 (predators \rightarrow herbivores), and 0.05 (herbivores \rightarrow predators). Intraspecific interactions were selected at random between -0.1 and -0.2 for plants, and set to -0.2 for herbivores and predators. For competitive communities, interspecific values of b_{ij} were selected uniformly between 0 and -0.1 , and intraspecific values between -0.06 and -0.16 . For the arbitrary topology, the probability of any pair of species interacting was 0.5, and of the interacting pairs of species 45% were competitors, 45% were prey and predators, and 10% were mutualists. The magnitudes of interspecific values of b_{ij} were selected uniformly between 0 and 0.1, with sign dictated by type of interaction, and intraspecific values were selected between -0.06 and -0.16 .

For the random food webs (Fig. 3), we selected intra- and interspecific values of b_{ij} from uniform $(-0.1, 0)$ and $(-0.1, 0.1)$ distributions, respectively. The interaction coefficients b_{ij} were then modified by multiplying interspecific coefficients by p . The vast majority of resulting food webs when $p > 0.5$ were unstable, but comparable analyses constrained to stable food webs gave similar results.

In selecting coefficients, we constrained values for intraspecific interactions b_{ii} to negative numbers. Otherwise, for the case of no interactions (or when communities are reduced to one species) intensifying the stressor increases species abundances. This is because in the absence of species interactions, $\delta_i(N) = -a_i/b_{ii}$, which is positive when $b_{ii} > 0$.

Values of $\delta_i(N)$ were calculated by solving the set of equations satisfied by equation (1) at equilibrium:

$$r_i + a_i s + \sum_j b_{ij} x_j^* (s) = 0 \quad (i = 1, \dots, N) \quad (3)$$

and new values of $\delta_i(N)$ were calculated sequentially as the community size was reduced. Our assumption that the species with the lowest tolerance $\delta_i(N)$ goes extinct first was supported by simulating the full model given by equation (2) (for example, Fig. 1a, b) and categorizing species as extinct once they were reduced in abundance by a factor of 10^{-3} .

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Resonance effects indicate a radical-pair mechanism for avian magnetic compass

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Migratory birds are known to use the geomagnetic field as a source of compass information^{1,2}. There are two competing hypotheses for the primary process underlying the avian magnetic compass, one involving magnetite^{3–5}, the other a magnetically sensitive chemical reaction^{6–8}. Here we show that oscillating magnetic fields disrupt the magnetic orientation behaviour of migratory birds. Robins were disoriented when exposed to a vertically aligned broadband (0.1–10 MHz) or a single-frequency (7-MHz) field in addition to the geomagnetic field. Moreover, in the 7-MHz oscillating field, this effect depended on the angle between the oscillating and the geomagnetic fields. The birds exhibited seasonally appropriate migratory orientation when the oscillating field was parallel to the geomagnetic field, but were disoriented when it was presented at a 24° or 48° angle. These results are consistent with a resonance effect on singlet–triplet transitions and suggest a magnetic compass based on a radical-pair mechanism^{7,8}.

The magnetic compass of birds is light-dependent^{9,10}, and exhibits strong lateralization with input coming primarily from the right eye¹¹. However, the primary biophysical process underlying this compass remains unexplained. Magnetite^{3–5,12} as well as biochemical radical-pair reactions^{7,8} have been hypothesized to mediate sensitivity to Earth-strength magnetic fields through fundamentally different physical mechanisms. In the magnetite-based mechanism, magnetic fields exert mechanical forces³. In the radical-pair mechanism, the magnetic field alters the dynamics of transitions between spin states, after the creation of a radical pair through a light-induced electron transfer. These transitions in turn affect reaction rates and products^{7,8}. Although in most radical-pair reactions the effects of Earth-strength magnetic fields are masked by stochastic fluctuations, model calculations¹³ show that such effects can be amplified beyond the level of stochastic fluctuations in specialized radical-pair receptor systems.

Exploiting the principles of magnetic resonance, we developed a diagnostic tool to identify a radical-pair process as the primary process for a physiological magnetic compass. No change in magnetic alignment of magnetite receptors is expected for weak oscillating fields with frequencies larger than 100 kHz (ref. 14).

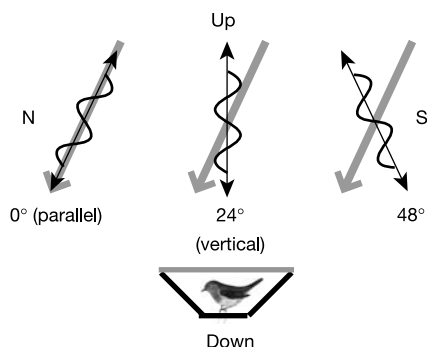


Figure 1 Experimental set-up. Orientation of the 7.0-MHz oscillating magnetic fields (black arrows with sine curve) parallel, at a 24° (vertical) and at a 48° angle to the geomagnetic field (grey arrows; see Fig. 2c–e for results). In the parallel and 48° conditions, the oscillating fields have the same angle with respect to the birds in our experimental set-up.

However, an oscillating magnetic field that is in resonance with the splitting between radical-pair spin states can perturb a radical-pair mechanism by directly driving singlet–triplet transitions. In typical biomolecules, many hyperfine splittings occur in the range of 0.1–10 MHz and a limited number may exist in the range of 10–25 MHz (ref. 15).

We used the migratory orientation of European robins, *Erithacus rubecula*, to detect the possible effects of oscillating magnetic fields on the underlying magnetoreception mechanism. Orientation tests were performed during spring migration under 565 nm light; conditions under which robins normally show excellent orientation using their inclination compass^{16,17}. All birds were tested indoors, in the local geomagnetic field of 46 μ T and 66° inclination. In addition to the control condition (geomagnetic field alone, no oscillating field), we used four experimental conditions in which an oscillating magnetic field was added to the geomagnetic field (Fig. 1).

In the control condition, the robins exhibited seasonally appropriate northerly orientation (Fig. 2a), but in the presence of broadband (0.1–10 MHz, 0.085 μ T) and single-frequency (7 MHz, 0.47 μ T) oscillating fields, both vertically aligned (see Fig. 1), the birds were disoriented (Fig. 2b, d).

To confirm that the observed behavioural change was caused by a direct effect of the oscillating fields on the magnetic compass and not by nonspecific effects due to changes in motivation and so on, we varied the alignment of the 7.0-MHz field. The frequencies at which an oscillating field perturbs a radical-pair reaction depend not only on the chemical nature of the radical pair, but also on the alignment of the oscillating field with respect to the static field¹⁸. This implies that the responses of a magnetic compass system based on radical pairs in the presence of a weak, single-frequency oscillating field can depend on the alignment of the oscillating field, whereas nonspecific effects should occur independently of alignment. We tilted the oscillating field 24° to the north or 24° to the south, so that the two oscillating fields were aligned at the same angle relative to the vertical, but at different angles, parallel and 48°, relative to the geomagnetic field (Fig. 1).

When the oscillating field was parallel to the geomagnetic field, the birds oriented in the migratory direction (Fig. 2c) and their response was indistinguishable from that of the control condition (Table 1). In contrast, when the same oscillating field was presented at 24° and 48° relative to the geomagnetic field, the birds were disoriented (Fig. 2d, e) and their response differed significantly from that of the control condition ($P < 0.01$). The intra-individual scatter in the distribution of nightly headings, as reflected by the length of the birds' mean vectors (r_b), was indistinguishable from that of the control condition when the 7-MHz oscillating field was parallel to the geomagnetic field, but was significantly greater (lower

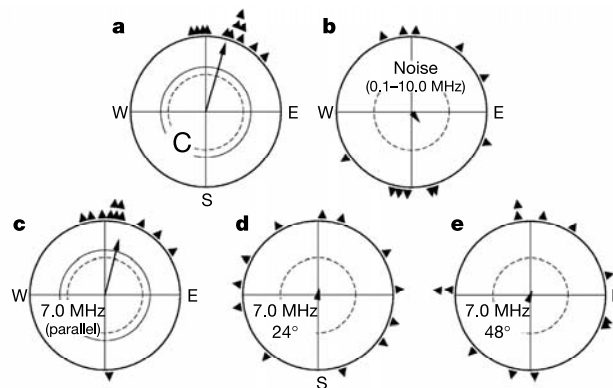


Figure 2 Effects of oscillating magnetic fields on magnetic orientation behaviour of European robins. Triangles indicate the mean headings of the 12 test birds, arrows represent the grand mean vectors (unit length = outer circle radius; see Table 1 for numerical values). The inner circles mark the 5% (dotted) and the 1% significance border of the Rayleigh test²⁷. **a**, Tests in the geomagnetic field only. **b**, Tests in the geomagnetic field and a broadband (0.1–10 MHz) noise field of 0.085 μ T. **c–e**, Tests in a 7.0-MHz field of 0.47 μ T, oriented either parallel (**c**), at a 24° angle (**d**), or at a 48° angle (**e**) to the geomagnetic field.

r_b) in the other three oscillating-field conditions (that is, broadband and 7 MHz at 24° and 48° angles) (see Table 1).

Our findings show that it is unlikely that oscillating fields have an effect on magnetite-based receptors^{3–5,12}, because the dampening effects of the cellular environment prevent magnetite particles from tracking weak radio-frequency magnetic fields. Even in very-low-viscosity physiological conditions (spherical single-domain magnetite in water) we can estimate, following ref. 14, that a 7-MHz field would require an intensity of 285 μ T to produce a noticeable change in alignment, which is far stronger than the 0.47 μ T fields used in our experiments. Likewise, frequencies used in these experiments of less than 10 MHz are far from the expected ferromagnetic resonance frequencies in the GHz range¹⁹, thus rendering thermal or lattice vibration effects of the oscillating fields on magnetite unlikely.

In contrast, resonance effects of oscillating magnetic fields in the frequency range of 0.1–10 MHz are expected in a radical-pair mechanism because hyperfine splittings occur in this range¹⁵. Resonance effects in this frequency range would also be expected in the context of Leask's optical pumping hypothesis⁶, although the lack of evidence for a biological source of energy in the radio-frequency range required by the optical-pumping process⁶ makes this mechanism unlikely.

By what physical mechanism could the remarkably weak oscillating fields used in our experiments (0.085 μ T, 0.47 μ T) affect a radical-pair reaction, and in turn, a radical-pair-based compass system? A simple model calculation (see Methods) suggests that at least in some radical-pair reactions (radical pairs with one dominant hyperfine interaction and a long lifetime), a resonant oscillating magnetic field of a thousandth of the geomagnetic field strength can produce a detectable effect. This remarkable sensitivity to very weak resonant oscillating fields is a noteworthy feature and further studies should analyse the limits of sensitivity in more realistic descriptions of radical pairs.

Our data, together with the above analysis, indicate that a magnetically sensitive radical-pair process in European robins is linked to the physiology of magnetic compass orientation. The most straightforward explanation for our findings is that the radical-pair process indicated by our experiments works as the primary process underlying magnetic compass orientation in European robins and probably in other birds¹⁰. Of course, we cannot exclude the possibility that a radical-pair reaction that is part of an unrelated biochemical pathway was affected. However, the fact that resonance

Table 1 Orientation of European robins in different oscillating magnetic field conditions

Bird	Geomagnetic field only		Noise (0.1–10 MHz)		7.0 MHz parallel		7.0 MHz 24°		7.0 MHz 48°	
	α_b	r_b	α_b	r_b	α_b	r_b	α_b	r_b	α_b	r_b
R 1	26°	0.98	339°	0.24	358°	1.00	110°	0.53	274°	0.45
R 2	20°	0.76	183°	0.42	4°	0.95	126°	0.48	9°	0.84
R 3	47°	0.91	194°	0.61	344°	0.70	86°	0.32	226°	0.98
R 4	350°	0.72	3°	0.21	10°	0.99	17°	0.37	32°	0.20
R 5	15°	0.94	189°	0.74	12°	0.99	162°	0.37	112°	0.85
R 6	1°	0.94	37°	0.90	27°	0.80	330°	0.29	351°	0.17
R 7	18°	1.00	64°	0.42	350°	0.29	297°	0.44	193°	0.73
R 8	20°	0.99	112°	0.51	57°	0.45	220°	0.96	109°	0.11
R 9	354°	0.97	354°	0.80	177°	0.27	58°	0.89	177°	0.32
R 10	24°	0.82	166°	0.09	8°	0.99	261°	0.42	352°	0.15
R 11	358°	0.81	163°	0.84	6°	0.99	278°	0.28	75°	0.80
R 12	37°	0.79	235°	0.47	41°	0.86	3°	0.78	273°	0.31
Grand mean vector	16°, 0.96***		142°, 0.18 ^{n.s.}		14°, 0.78***		11°, 0.10 ^{n.s.}		22°, 0.07 ^{n.s.}	
Median individual vector length	0.93		0.49		0.90		0.43		0.38	
ΔC	C	C	**	***	n.s.	n.s.	**	***	**	**

The α_b and r_b values are based on three recordings of the bird under the respective condition. The grand mean vector is given with its significance by the Rayleigh test indicated by asterisks, followed by the median individual vector length. The bottom line indicates significant differences from the control data obtained in the geomagnetic field only (see Methods for tests). Significance levels: ***, $P < 0.001$; **, $P < 0.01$; n.s., not significant.

effects are only expected in specialized radical-pair systems that can also detect the geomagnetic field^{7,13}, makes it unlikely that a radical-pair process not associated with magnetoreception was affected. There is currently no evidence supporting the existence of such a magneto-sensitive radical-pair process outside the context of magnetoreception and even if one existed, it is uncertain whether it would affect orientation behaviour. In our study we observed no change in activity between birds in oscillating-field and control conditions; and food intake and the general appearance of the birds was normal, suggesting that their health and motivation were unaffected by the brief 75 min exposure to oscillating magnetic fields. In view of this, our findings probably reflect a direct effect of the oscillating fields on the compass mechanism.

This conclusion does not rule out the possibility that birds possess an additional magnetically sensitive system based on magnetite. Magnetite in the form of single domains and super-paramagnetic crystals embedded in specialized structures has been described in the ethmoid region and in the upper beak of migratory birds and pigeons^{20,21}. However, behavioural evidence^{22–24} as well as electrophysiological recordings^{25,26} suggest that the magnetite discovered is not involved in magnetic compass orientation, but instead forms the basis of a magnetic-intensity sensor, potentially used in a magnetic 'map' sense for determining geographic position.

Our study establishes the use of oscillating magnetic fields as a diagnostic tool that can indicate the involvement of a magneto-sensitive radical-pair reaction in birds. Extending this tool to determine the frequency range in which oscillating fields affect the radical-pair mechanism can reveal the chemical nature of the radical pairs involved. Finally, using oscillating magnetic fields as a diagnostic tool is not specific to birds and should be easily transferable to assays with other animal groups. The threshold intensity at which oscillating-field effects can be observed provides information about the underlying mechanism. Behavioural effects from oscillating fields of similar intensity to those used in the present study would suggest a radical-pair mechanism. The absence of behavioural effects from oscillating fields of intensities greater than 50 μ T would make a radical-pair mechanism unlikely. □

Methods

Test birds

European robins are small passerines that migrate at night. The test birds were mist-netted as transigrants in early September 2002 in the Botanical Garden near the Zoological

Institute in Frankfurt am Main (50° 08' N, 8° 40' E). The birds were kept indoors in individual cages over the winter on a photoperiod that simulated local conditions until December, but was then reduced to 8:16 h light:dark. In the beginning of January 2003, the photoperiod was increased to 13:11 h light:dark. This induced premature *Zugunruhe* (nocturnal migratory restlessness); the experiments took place between 13 January and 17 February 2003.

Test conditions

The tests took place in wooden huts in the garden of the Zoological Institute within the local geomagnetic field of 46 μ T and 66° inclination. To produce the oscillating fields, we modified a test design developed by J.B.P. for similar tests (J.B.P., unpublished), consisting of a coil antenna (210 cm diameter) mounted on a rotatable wooden frame surrounding the test arena. Oscillating currents from a high frequency (HF) generator (Stanford Research Systems DS 34) were amplified by a HF amplifier (Research AF Model 25 W 1,000) and fed into the coil through a resistance of 51 Ω . The coil consisted of a single winding of coaxial cable (RG62A/U, 93 Ω) with 2 cm of the screening removed opposite the feed. The HF field was measured daily, before each test session, using a spectrum analyser (HP89410A) and a magnetic field probe (Rohde & Schwarz, HZ-11816.2770.0, 3 cm probe). Within the test arena, the inhomogeneity of the field was less than 15%. Variations in field intensities between tests were less than 20% of the average value. The HF generator and amplifier were placed outside the huts in varying positions with respect to the test arena. They were switched on during the majority of control tests, but with the power generator turned to zero; comparisons with control tests without this arrangement revealed no observable effect of this procedure.

Test apparatus and procedure

Testing followed standard procedures¹⁶: birds were tested individually in funnel-shaped PVC cages (35 cm upper diameter; 20 cm high) lined with coated paper (BIC Germany, formerly TippEx); the birds left scratches in the coating as they moved. The cages were covered with an opaque plexiglass cover and placed in PVC cylinders, the top of which consisted of a plastic disk carrying the same green light-emitting diodes as those used in earlier studies^{9,16} (peak frequency at wavelength $\lambda = 565$ nm, with $\lambda/2$ at 533 and 583 nm, respectively). The light passed through two diffusers before reaching the bird with an intensity of 2.1 mW m⁻².

The birds were tested once per day. Tests began when the light went out in the housing cages and lasted about 75 min. Each bird was tested three times in each condition. The three tests were arranged in sets; the set of second and third tests began after the set of first and second tests respectively was completed. Within each set, the tests in the various conditions were performed in a pseudo-random order, with the sequence differing between birds.

Data analysis and statistics

For the data analysis, the coated paper was divided into 24 sectors, and the scratches per sector were counted by experimenters that were blind to the test condition. The heading of the respective test was determined by vector addition. From the three headings per test condition for each bird, the mean vector with heading α_b and length r_b was calculated. The twelve α_b values were combined to a grand mean vector, which was tested for directional significance using the Rayleigh test²⁷. The distributions of the birds' α_b values in different conditions were compared using the Mardia-Watson-Wheeler test²⁷. The r_b values, representing the intra-individual variance in locating the migratory direction, are not normally distributed; and so medians are given for each test condition. The r_b values were compared with those obtained under the control conditions using the Wilcoxon test for matched pairs of data.

Model calculations

We used a one-proton radical-pair model²⁸ with an isotropic hyperfine coupling, a , of 0.5 mT, an anisotropy, α of 0.3, and a lifetime of 20 μ s (corresponding to the observed lifetime of flavin-tryptophan radical pairs¹⁵). We solved the stochastic Liouville equation to determine the triplet yield in the presence of a static magnetic field of 46 μ T. We then calculated, by direct numerical integration of the stochastic Liouville equation, the change in triplet yield, $\Delta\Phi_{\text{OMF}}$, caused by an additional 1.3 MHz oscillating magnetic field in resonance with the splitting due to the 46 μ T static field. For comparison, we also calculated the triplet yield change, $\Delta\Phi_{\text{static}}$, resulting from a decrease of 12 μ T in static field, noting that such a change led to disorientation in the magnetic compass orientation responses of robins²⁹. The intensity of the oscillating field required for $\Delta\Phi_{\text{OMF}}$ to equal $\Delta\Phi_{\text{static}}$ is 0.033 μ T, that is, less than any of the intensities employed in our experiments.

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Modelling disease outbreaks in realistic urban social networks

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Most mathematical models for the spread of disease use differential equations based on uniform mixing assumptions¹ or *ad hoc* models for the contact process^{2–4}. Here we explore the use of dynamic bipartite graphs to model the physical contact patterns that result from movements of individuals between specific locations. The graphs are generated by large-scale individual-based urban traffic simulations built on actual census, land-use and population-mobility data. We find that the contact network among people is a strongly connected small-world-like⁵ graph with a well-defined scale for the degree distribution. However, the locations graph is scale-free⁶, which allows highly efficient outbreak detection by placing sensors in the hubs of the locations network. Within this large-scale simulation framework, we then analyse the relative merits of several proposed mitigation strategies for smallpox spread. Our results suggest that outbreaks can be contained by a strategy of targeted vaccination combined with early detection without resorting to mass vaccination of a population.

The dense social-contact networks characteristic of urban areas form a perfect fabric for fast, uncontrolled disease propagation. Current explosive trends in urbanization exacerbate the problem: it is estimated that by 2030 more than 60% of the world's population will live in cities⁷. This raises important questions, such as: How can an outbreak be contained before it becomes an epidemic, and what disease surveillance strategies should be implemented? Recent studies¹, under the assumption of homogeneous mixing, make the case for mass vaccination in response to a smallpox outbreak. With different assumptions, it has been shown² that mass vaccination is not required. Policymakers must trade off the risks associated with vaccinating a large population⁸ against the poorly understood risks of losing control of an outbreak. Addressing such specific policy questions⁹ requires a higher-resolution description of disease spread than that offered by the homogeneous-mixing assumption and the differential-equations approach.

Here we present a highly resolved agent-based simulation tool (EpiSims), which combines realistic estimates of population mobility, based on census and land-use data, with parameterized models for simulating the progress of a disease within a host and of transmission between hosts¹⁰. The simulation generates a large-scale, dynamic contact graph that replaces the differential equations of the classic approach. EpiSims is based on the Transportation Analysis and Simulation System (TRANSIMS) developed at Los Alamos National Laboratory, which produces estimates of social networks based on the assumption that the transportation infrastructure constrains people's choices about where and when to perform activities¹¹. TRANSIMS creates a synthetic population endowed with demographics such as age and income, consistent with joint distributions in census data. It then estimates positions

Responses of neurons to an amplitude modulated microwave stimulus

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Abstract

In this study we investigated the effects of a pulsed radio frequency signal similar to the signal produced by global system for mobile communication telephones (900 MHz carrier, modulated at 217 Hz) on neurons of the avian brain. We found that such stimulation resulted in changes in the amount of neural activity by more than half of the brain cells. Most (76%) of the responding cells increased their rates of firing by an average 3.5-fold. The other responding cells exhibited a decrease in their rates of spontaneous activity. Such responses indicate potential effects on humans using hand-held cellular phones.

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Keywords: Cellular telephone; Magnetic field; Health risk; Avian; Central nervous system

The postulated biological effects of electromagnetic fields are highly diverse, ranging from use of natural fields by animals for navigation to thermal cooking that occurs with strong fields such as those produced by microwave ovens [7]. It has been shown that even the weak fluctuations of Earth-strength magnetic fields influence the electrical activity of neurons and pineal cells and the synthesis of melatonin in birds and mammals [1,9,10], including humans [6]. Athermal effects have been the most difficult to explain because the mechanism by which they affect biological tissue is usually unknown. The question arises as to whether there is a particular sensitivity of the neural tissues of the brain to high frequency electromagnetic fields such as is produced by broadcast transmitters.

We tested the effects of electromagnetic radio frequency (RF) signals having a carrier frequency of 900 MHz, unmodulated and pulse modulated at 217 Hz with a duty cycle of 12.5% and a peak power density of 0.1 mW/cm². This stimulus was selected because it is similar to that used by the global

system for mobile communication (GSM) telephone system. The calculated average specific absorption rate (SAR) of this stimulus for the test subjects was 0.05 W/Kg, based on the equations in Durney and coworkers [8]. The test subjects were 34 adult zebra finches (*Taenopygia guttata*), anesthetized with a mixture of ketamine (0.05 mg/g) and xylazine (0.01 mg/g) injected i.m. into the pectoralis major. The anesthetized bird was mounted in a nonconducting plastic holder. The bird and the holder were placed inside a tuned RF cavity (23.5 cm diameter, 100.5 cm long) made of perforated metal. We used a resonant cavity (length = 3λ) because the resulting electrical field was a standing wave and, therefore, was uniformly distributed within the cavity and was measured accurately at the demodulating stub. The resonant cavity was fitted with two tuned RF stubs (each 16.5 cm [$\lambda/2$] from opposite ends): one for emitting the signal and one for monitoring the frequency and power of the signal within the cavity. This arrangement resulted in the two stubs being 2λ from each other causing the signal at the demodulation stub to be synchronized in phase and intensity to the emitted signal. The entire bird was within the cavity and positioned such that the bird's head was at the center of the cavity. This position put the bird's head exactly 1λ from the emitting stub and the demodulating stub. Consequently, the signal the bird's head received was exactly the signal at both of those locations. To record from neurons in the brain of the bird, a small hole (4 mm diameter) was made through the skull. A glass microelectrode (tip diameter 1–2 μ m) filled with a conducting solution

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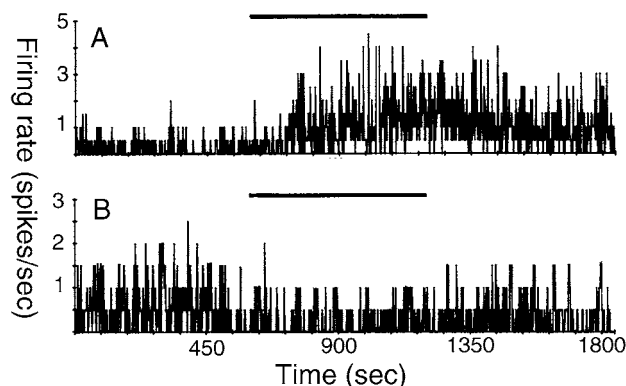


Fig. 1. Examples of neuronal responses in the zebra finch brain to stimulation of a 217 Hz, 12.5% duty cycle square wave modulated 900 MHz carrier signal: (A) simulation and (B) inhibition. The solid bar above each graph indicates the presence of the stimulating RF signal.

of physiological saline, to reduce conductivity, was slowly advanced into the brain through this hole until a spontaneously active nerve cell was detected. A silver reference electrode was inserted beneath the skin along the back of the head directly behind the glass microelectrode to complete the circuit. Arranging the electrodes along the long axis of the cavity prevented them from acting as a loop antenna and electrically stimulating the cells. Once a spontaneously active cell was located, it was tested with the stimulus. The protocol for all the testing procedures was a 10 min prestimulus period, a 10 min stimulus period, and a 10 min poststimulus period. The rates of the cell's activity during these three time intervals were compared to detect any effect of the stimulation. A responding cell was one that changed its firing rate during the stimulation by at least 10%.

The microwave stimulus signal was produced by an Amplifier Research amplifier (model 10W1000M7) driven by an HP 8350A sweep oscillator with an HP 83522A RF unit set for 900 MHz. Amplitude modulation of the signal was produced by a free running HP 3314A function generator set for 217 Hz square wave signal with a duty cycle of 12.5%. The output of the amplifier was switched between a matched load and the cable to the waveguide chamber by a single-pole, double-throw RF switch (HP 8761A). The switch was controlled by a digital signal from the computer program TIDA on an IBM-compatible microcomputer. The frequency and intensity of the emitted signal were monitored using an HP 5342A microwave frequency counter connected to the demodulator stub in the waveguide cavity. All power measurements were of peak power.

We recorded 133 spontaneously active units from 34 anesthetized adult zebra finches. The recording locations were in the cerebrum (Pars occipitalis and Pars parietalis) and Folia of the anterior cerebellum. Ninety-one units (69%) showed some response to the stimulation: 69 (52%) responded with excitation (Fig. 1A) and 22 (17%) responded with inhibition (Fig. 1B). The remaining 42 (31%) cells showed no discernible response. The cells

showing excitation responded with increases in their rate of firing to the stimulation (mean rate during stimulation = 3.5 ± 0.30 [SE] times prestimulus rate; Fig. 2). Most of the inhibitory responses were small (mean rate during stimulation = 0.4 ± 0.07 times prestimulus rate; Fig. 2), in part because the cells were firing slowly before the stimulation. There was a significant difference among the firing rates of the three responses and the prestimulus firing rate (Kruskal–Wallis test: $H_c = 216.8$, $P < 0.001$, $\nu = 5$; see Fig. 3). Based on a non-parametric multiple comparison [13], the firing rates in the three response categories different from one another significantly ($P < 0.05$; $Q = 3.817$ – 4.341). There was no significant difference among the firing rates of the nonresponding cells during the prestimulus, stimulus, and post-stimulus periods ($P > 0.05$). All responses we recorded were to power densities of 0.1 mW/cm^2 ($\text{SAR} = 0.05 \text{ W/Kg}$) and stronger (up to 0.5 mW/cm^2). The mean latency from the initiation of the stimulus to the start of the response was $104 \pm 197 \text{ s}$, with the response lasting beyond the end of the stimulus period in half of the responding cells. The mean persistence beyond the end of stimulation was $308 \pm 68 \text{ s}$, but there was no correlation ($r = 0.489$, $P > 0.05$) between the latency of the response and how long the cell continued responding beyond the end of the stimulus.

Three cells that responded to the modulated carrier were also tested with an unmodulated signal of the same carrier frequency. The power of the unmodulated signal was tested at two densities: one that equaled the peak power of the modulated stimulus and one that equaled the average power of the modulated stimulus. None of these cells exhibited a response to the unmodulated carrier. In addition to responses to the nominal stimulus, we also tested four cells that did not respond to the 0.1 mW/cm^2 pulsed signal with higher power densities

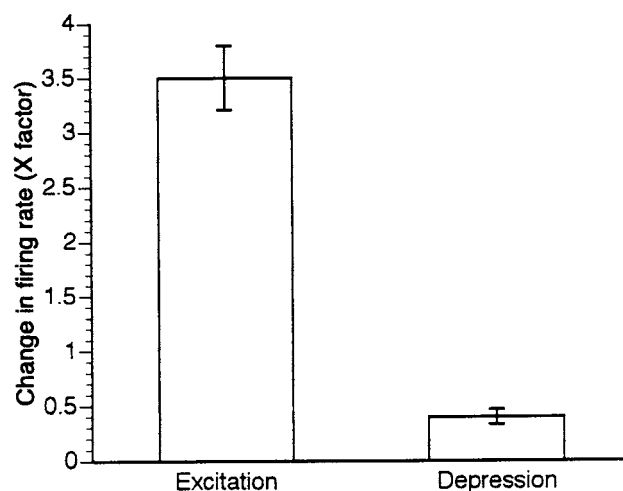


Fig. 2. Mean relative firing rates of cells that responded to the simulated GSM signal and were categorized as excitation or depression. The firing rates are relative to the cells' firing rates during the prestimulus period. The vertical bars indicate 1 standard error.

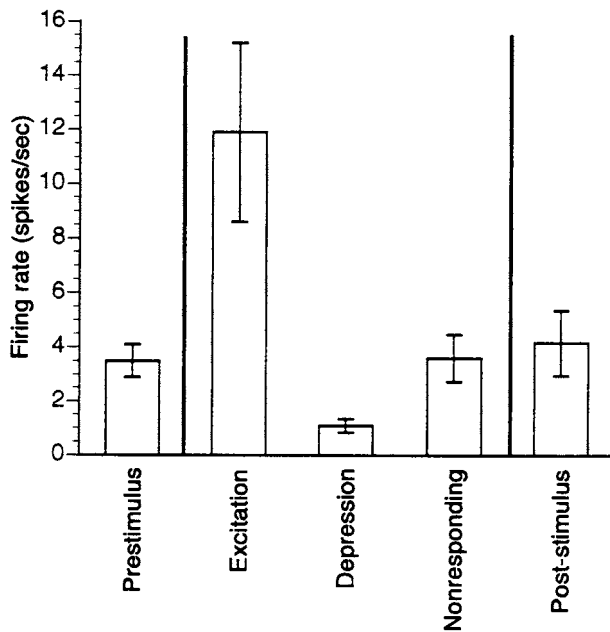


Fig. 3. Firing rates of zebra finch neurons during the prestimulus (10 min), stimulus (10 min), and poststimulus (10 min) periods. The poststimulus values are for the non-responding cells only because the responding cells often continued their response into the post-stimulus period (see text for details). The firing rates of the three responses differed significantly (Kruskal-Wallis test: $H_c = 216.8$, $P < 0.001$, $\nu = 5$). The vertical bars indicate 1 standard error.

(up to 0.5 mW/cm^2). Three cells did not respond to the stronger intensities, but one cell that did not respond to the 0.1 mW/cm^2 stimulus responded to an intensity of 0.3 mW/cm^2 with depression of its rate of activity.

One concern was that the electrodes themselves were acting as an antenna and stimulating the cells electrically. The arrangement of the active and reference electrode centered along the long axis of the waveguide chamber prevented them from serving as a loop antenna. In preliminary experiments we varied the positions of the electrodes to determine whether they could, in fact, act as an antenna. When the electrodes were not aligned longitudinally, the stimulus artifact was detected directly and observed on the oscilloscope display. Whether such a stimulus was strong enough to stimulate the cells is unknown. A second factor that supports the idea that the cells were not stimulated electrically is that not all cells responded to the stimulus, even those in the close neighborhood of a responding cell. This clearly speaks against an artifact.

These high frequency RF fields produced a response in many types of neurons in the avian central nervous system (in both cerebellum and cerebrum) and did not appear to be limited to any specialized receptor. Similar responses (long latency and ongoing higher activity after cessation of the stimulus) also were recorded to a 52 GHz carrier, 16 Hz modulated signal (Semm et al., unpubl. data). Thus, the effect does not appear to be limited to magnetic sensory cells [11], but may occur in any part of the brain. The similar

responses to different frequencies point toward a common mechanism of low frequency modulation, perhaps at the cell membrane. Such a stimulus might mimic a natural mechanism involved in cell communication, producing responses from many different types of neurons. It is unlikely that the effects we observed are the result of thermal excitation caused by the RF radiation because the power densities we applied were 2–3 orders of magnitude below what is required (10 mW/cm^2) to produce heating of even 0.5°C [2]. It is also unlikely that localized areas of the brain were heated and thermally stimulated because neurons responded only to the modulated signal and did not respond to unmodulated signals that were the same strength. Consequently, we conclude that the effects we observed are not the result of thermal agitation but at this point we cannot offer an athermal mechanism to account for the observations.

Although individual neurons in the zebra finch brain responded to the pulsed RF stimulus, we do not know whether these responses by the nervous system are manifested in the bird's behavior or its health. Bruderer and coworkers [4,5] reported no behavioral responses of birds to pulsed or continuous RF microwave signals, but their studies involved different frequencies and lower power densities of the stimulus. Thuróczy and coworkers reported neuronal responses of freely moving rats [12] similar to the responses we observed in the zebra finch. During the period of stimulation, sensitive cortical neurons of Long Evans rats showed either an increase or a decrease in the rate of spontaneous activity. The changes in firing rates were less than the changes we observed in the zebra finch: an increase of less than $2\times$ in the rat versus $3.5\times$ in the finch and a decrease to $0.67\times$ in the rat versus $0.4\times$ in the finch. Although the neuronal responses were similar between the rat and the finch, the SAR values of the RF field used with the rat were much greater than that used for the finch. Thuróczy and coworkers also observed behavioral responses by the rat to the GSM signal. In conditioning experiments, the rats' reaction times decreased during stimulation as did their learning rate (as measured by discrimination tasks).

Whether similar neuronal responses occur in other mammals, including humans, requires further investigation. Borbély and coworkers [3] reported that exposure to a RF signal similar to the one we used influenced sleep and sleep electroencephalogram in humans. Their results and the responses we recorded clearly indicate the potential for effects on the human nervous system.

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Possible Effects of Electromagnetic Fields from Phone Masts on a Population of White Stork (*Ciconia ciconia*)

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Monitoring of a white stork population in Valladolid (Spain) in the vicinity of Cellular Phone Base Stations was carried out, with the objective of detecting possible effects. The total productivity, in the nests located within 200meters of antennae, was 0.86 ± 0.16 . For those located further than 300m, the result was practically doubled, with an average of 1.6 ± 0.14 . Very significant differences among the total productivity were found ($U = 240$; $p = 0.001$, Mann-Whitney test). In partial productivity, an average of 1.44 ± 0.16 was obtained for the first group (within 200m of antennae) and of 1.65 ± 0.13 for the second (further than 300m of antennae), respectively. The difference between both groups of nests in this case were not statistically significant ($U = 216$; $P = 0.26$, Mann-Whitney Test U). Twelve nests (40%) located within than 200m of antennae never had chicks, while only one (3.3%) located further than 300m had no chicks. The electric field intensity was higher on nests within 200m (2.36 ± 0.82 V/m) than on nests further than 300m (0.53 ± 0.82 V/m). Interesting behavioral observations of the white stork nesting sites located within 100m of one or several cellsite antennae were carried out. These results are compatible with the possibility that microwaves are interfering with the reproduction of white storks and would corroborate the results of laboratory research by other authors.

Keywords Cellsites; Cellular phone masts; *Ciconia ciconia*; Electromagnetic fields; Microwaves; Nonthermal effects; Reproduction; White stork.

Introduction

Most of the attention on the possible biological effects of electromagnetic fields (EMF) has been focused on human health. People frequently use wildlife as biological indicators to detect the alterations in the ecosystems and in an urban

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habitat. The numeric tendency of the populations of birds is of particular interest in the conservation of nature [1].

The cellsite antennae emit a frequency of 900 or 1800 MHz, pulsed in very low frequencies, generally known as microwaves (300 MHz–300 GHz), similar to the radar spectrum. The cellsite ordinarily have 3 sectors, with 3 antennae that cover an angle of 120 degrees each [2–5]. Though they have many and varied outputs, at a distance of 50 m, the power density is about $10 \mu\text{W}/\text{cm}^2$ [2], while at distances of 100 m at ground level it measures above $1 \mu\text{W}/\text{cm}^2$ (personal observation). Between 150 and 200 m, the power density of the main lobe near the ground is typically of some tenth of $1 \mu\text{W}/\text{cm}^2$ [3].

In real life, living organisms are exposed to variable levels of electromagnetic fields (radiofrequencies), according to the distance from the cellular bases stations, the presence of passive structures to either amplify the waves (e.g., the metallic structures) or to shield them (buildings or other obstacles), the number of transmission calls within the transmitters and their position with relationship to the orientation of the antenna [2].

Animals are very sensitive electrochemical complexes that communicate with their environment through electrical impulses. Ionic currents and electric potential differences exist through the cellular membranes and corporal fluids [6]. The intrinsic electromagnetic fields from the biological structures are characterized by certain specific frequencies that can be interfered with by the electromagnetic radiation, through induction and causing modification in their biological responses [3]. Animals exposed to the EMF can suffer a deterioration of health, changes in behavior [7, 8], and changes in reproductive success [9, 10].

The low intensity pulsed microwave radiation from cellsites produces subtle athermal influences in the living organisms, because this radiation is able to produce biological responses by the microwave carrier and by the low frequency of pulses from GSM system. “Windows” exist in whereby EMFs produce biological effects at specific frequencies (window effect) [11]. Some effects are manifested exclusively with a certain power density [12], while others are manifested after a certain duration of the irradiation, which indicates long-term cumulative effects [13]. During lingering exposure, the effects can change from stimulant to inhibition, depending on the pulse shape [14, 15], the duration, development, and differentiation and the physiologic condition or health of the receiving organism [16], and their genetic predisposition [17]. These waves seem to cause different, and even contrary effects, depending on their frequency, intensity, modulation, pulses or time of exposure [12, 16, 18]. The pulsed waves (in bursts) and certain low frequency modulations, produce great biological activity [14, 15, 18]. The dose-response relationships (athermal) are nonlinear [19].

Research has shown such effects on the living organisms at molecular [12] and cellular levels [20] on immune processes [21], in DNA [22], on the nervous, cardiac, endocrine, immune, and reproductive systems [16, 23–28], modification of sleep and alteration of the cerebral electric response (EEG) [29], increase of the arterial pressure and changes in the heart rhythm [30], and an increase in the permeability of the blood brain barrier [31].

The objective of this study was to investigate if the phone mast cellsites caused effects in wild birds similar to the laboratory studies, and studies carried out on people exposed to this radiation [3, 5, 32–35].

Materials and Methods

For monitoring the breeding success of the white stork population, nests ($n = 60$) were selected and visited from May to June of 2003. The difficulty of the investigation in the field, (and when studying wild species) does not allow one to control all variables as in the laboratory; however, the selected nests had similar characteristics. They were located in the roof of churches and buildings inside urban nuclei in Valladolid (Spain). (The nests on trees and other natural supports or outside the urban nuclei were never studied.) Since the cellsite radiations are omnipresent, very few places exist with an intensity of 0 V/m near inhabited nuclei. For that reason, nests were chosen that were exposed at very high or very low levels of electromagnetic radiation, depending on the distance from the nests to the antennas.

The nests were selected and separated in two categories:

- a) Nests ($n = 30$) located within 200m of one or several cellsite antennae (GSM-900 MHz and DCS-1800 MHz), placed in masts and in the roof of the buildings at 15–30m high.
- b) Nests ($n = 30$) located further than 300m of any cellsites.

The nest were observed using a prismatic Zeiss 8×30 and a “Leika” 20-60 X telescope. The number of young were counted.

For the analysis of the results of the reproduction, two indexes were used:

- 1) the total productivity (number of young flown by each couple, including nests with zero chicks).
- 2) the partial productivity (number of young flown by couples with some chicks, excluding nests with zero chicks).

To compare the breeding success of both groups of nests a nonparametric test was applied (Mann-Whitney test U).

Also, we measured the electric field intensity (radiofrequencies and microwaves) in V/m, using a “Nuova Elettronica” device Model LX 1435 with 10% sensitivity, from a unidirectional antenna (range: 1 MHz–3 GHz). Keeping in mind the inaccessibility of the nests, the measurements were made in their vicinity under similar conditions, recording the reproducible values obtained when directing the antenna of the device toward the cellsite antenna in line of sight.

Between February 2003 and June 2004, we carried out 15 and 10 visits, respectively, to 20 nests located within 100m of one or several cellsite antennae to observe the behavior of the species. The visits covered all the phases of breeding, from construction of the nest, until the appearance of young storks exercising their wings and practicing flight.

Results

Table 1 presents the number of young and electric field intensity (V/m) of each studied nest.

The total productivity, in the nests located within 200m of antennae was 0.86 ± 0.16 . For those located further than 300m, the result was practically doubled, with an average of 1.6 ± 0.14 (Table 1). Both groups showed very significant differences in the breeding success ($U = 240$; $P = 0.001$, Mann-Whitney Test U).

Table 1

Intensity of electric field, total and partial productivity in the nests within 200 m and further than 300 m to the phone mast

Nests within 200 m			Nests further than 300 m		
Nest	Number of young	EMF (V/m)	Nest	Number of young	EMF (V/m)
1	2	0.8	1	1	0.4
2	2	0.6	2	2	0.7
3	0	0.8	3	1	1.3
4	3	1.5	4	1	1.1
5	1	1.7	5	1	0.6
6	2	2.9	6	3	0.4
7	1	3.1	7	2	0.6
8	1	1.3	8	2	0.7
9	1	1.3	9	3	0.6
10	1	2.8	10	1	0.7
11	1	1.8	11	2	0.8
12	3	3.2	12	2	0.3
13	1	1.6	13	3	0.1
14	0	2.7	14	1	0.6
15	0	2.3	15	2	0.5
16	0	2.7	16	3	0
17	0	2.5	17	2	0.3
18	0	3.5	18	1	0.8
19	0	3.5	19	2	0.2
20	0	2.7	20	0	0.8
21	0	2.9	21	2	0.2
22	2	3.2	22	1	0.6
23	0	2.5	23	1	0.5
24	1	2.6	24	1	0.7
25	1	2.4	25	1	1.4
26	0	2.2	26	2	0.1
27	1	2.6	27	1	0.1
28	1	3.1	28	2	0.2
29	1	3.1	29	1	0
30	0	3.0	30	1	0.6
Mean EMF		2.36			0.53
Total productivity		0.86			1.6
Partial productivity		1.44			1.65
Nests without young		12 (40%)			1 (3.3%)

In partial productivity in average of 1.44 ± 0.16 was obtained for the first group (within 200 m of antennae) and 1.65 ± 0.13 for the second (further than 300 m of antennae) respectively. The difference between both groups of nests in this case was not statistically significant ($U = 216$; $P = 0.26$, Mann-Whitney Test U).

Twelve nests (40%) located within 200m of the antennae never had any chicks, while only one (3.3%), located further than 300m, never had chicks.

The electric field intensity was higher on nests within 200m (2.36 ± 0.82 V/m) than on nests further 300m (0.53 ± 0.82 V/m) (Table 1).

The results of the findings and interesting behavioral observations of the white stork nesting sites located within 100m of one or several cellsite antennae and on those that the main beam impacted directly ($EFI > 2$ V/m) included young that died from unknown causes. Also, within this distance, couples frequently fought over the nest construction sticks and failed to advance the construction of the nests. (Sticks fell to the ground while the couple tried to build the nest.) Some nests were never completed and the storks remained passively in front of cellsite antennae.

Discussion

The effects of athermal microwaves on birds have been well known for more than 35 years [36, 37]. Some authors obtained beneficial effects in the production of insect eggs and exposed birds, but found that the mortality was doubled [38]. In hen experiments, problems of health and a deterioration of the plumage arose, while in the autopsies, leucosis and tumors of the central nervous system appears [39]. Giarola and Krueger [40] obtained a large reduction of the rate of growth and also a reduction of the adrenal glands, in exposed chickens. Kondra et al. [41] obtained an increase in the frequency of ovulation of exposed birds, and a bigger production of eggs but with less weight, proposing that the pituitary gland was stimulated. Other authors also have obtained effects reducing the rate of growth in chickens and rats, reduction in the production of eggs in hens exposed to microwaves of different frequencies and intensities, increase of fertility, and a deterioration of the quality of the eggshell at certain frequencies [42]. An increase in the embryonic mortality of chickens also has been found [15, 17, 43, 44]. These microwave effects are athermal [45]. Recently, it also has been demonstrated that the microwaves used in cellphones produce an athermal response in several types of neurons of the nervous system in birds [46] and that they can affect the blood brain barrier as has been observed in rats [47].

Birds are especially sensitive to the magnetic fields [48]. The white stork (*Ciconia ciconia*) build their nests on pinnacles and other very high places with high electromagnetic contamination (exposed to the microwaves). Also, they usually live inside the urban environment, where the electromagnetic contamination is higher, and remain in the nest a lot of the time, for this reason the decrease on the brood can be a good biological indicator to detect the effects of these radiations.

The results indicate a difference in total productivity but not in partial productivity between the near nests and those far from the antennae. This indicate the existence of nests without chicks, or the death of young in their first stages in the nests near cellsites (40% of nest without young, compared to 3.3% in nests further 300m). Also, in the monitoring of the nests near to cellsite antennae, some dead young were observed and several couples never built the nest.

In previous studies in Valladolid, the results of productivity were generally higher than those obtained in this study and less nests appeared without young (Table 2).

Consistent with these results, the microwaves could be affecting one or several reproductive stages: the construction of the nest, the number of eggs, the embryonic

Table 2
Results of censuses carried out in Valladolid (Spain).

Year	Number of visited nests	Total productivity	Partial productivity	Couples without young(%)	References
1984	113	1.69	2.13	7	[65]
1992	115		1.93	5.2	[62]
1994	24	1.84		7.6	[63]
2001	35		2.43		[64]
2003 (<200m)	30	0.83	1.44	40	This study
2003 (>300m)	30	1.6	1.65	3.3	This study

development, the hatching or the mortality of chicks in their first stages. The faithfulness of the white stork to nest sites can increase the effects of the microwaves. A Greek study [49] relates to a progressive drop in the number of births of rodents. The mice exposed to $0.168 \mu\text{W}/\text{cm}^2$ become sterile after 5 generations, while those exposed to $1.053 \mu\text{W}/\text{cm}^2$ became sterile after only 3 generations. The interaction seems to take place through the central nervous system more than on the reproductive gland directly. Other studies find a decrease of fertility, increase of deaths after the birth in rats and dystrophic changes in their reproductive organs [16]. A recent study shows a statistically significant high mortality rate of chicken embryos subjected to the radiation from a cellphone, compared to the control group [43]. EMF exposure affected the reproductive success of kestrels (*Falco sparverius*), increasing fertility, egg size, embryonic development and fledging success but reduced hatching success [10]. An increase in the mortality [50] and the appearance of morphological abnormalities, especially of the neural tube [14, 15, 17] has been recorded in chicken embryos exposed to pulsed magnetic fields, with different susceptibility among individuals probably for genetic reasons. It is probable that each species, even each individual, shows different susceptibility to the radiation, since the susceptibility depends on the genetic bias, and of the irradiated living organisms physiologic and neurological state [4, 51]. Different susceptibility of each species also has been proven in wild birds exposed to CEM from high-voltage powerlines [9]. When the experimental conditions (power density, frequency, duration, composition of the tissue irradiated, etc.) change, their biological effects also change [25, 52]. Microwaves have the potential to induce adverse reactions in the health of people [2–5, 34, 35, 47]. Although the power output differs per site and type of transmitter, at more than 300m distance from the antennas, most of the symptoms recorded in people diminish or disappear [34, 35]. It also has been pointed out that below 0.6 V/m the effects on the people disappear (Salzburg resolution).

Since, we cannot see symptoms for white storks, it is necessary to use objective variables such as the Total and Partial Productivity, and other characteristics of behavior (nonconstruction of nest, sticks fall, etc.). We recommend electromagnetic contamination in the microwave range be considered a risk factor in the decline of some populations, especially urban birds, especially when exposed to higher radiation levels. Because of their thinner skull, their great mobility and the fact that they use areas with high levels of microwave electromagnetic radiation, birds

are very good biological indicators. The freedom of movement of birds and their habit of settling in the proximity and even on the cellsites, makes them potentially susceptible to such effects. Small organisms (children, birds, small mammals, etc.) are especially vulnerable, as absorption of microwaves of the frequency used in mobile telephones is greater as a consequence of the thinner skull of a bird, the penetration of the radiation into the brain is greater [2, 49, 53, 54].

Several million birds of 230 species die annually from collisions with the masts of telecommunication facilities in United States during migration [55]. The cause of the accidents has yet to be proven, although one knows that they mainly take place during the night, in fog, or bad weather. The birds use several orientation systems: the stars, the sun, the site-specific recognition and the geomagnetic field [48]. The illumination of the towers probably attracts the birds in the darkness, but it is possible that the accidents take place in circumstances of little visibility, because at the time, other navigational tools are not available. The perception to the terrestrial magnetic field can be altered by the electromagnetic radiation from the antennae. The reports of carrier pigeons losing direction in the vicinity of cellsites are numerous, and more investigation is necessary.

In the United Kingdom, where the allowed radiation levels are 20 times higher than those of Spain, a decline of several species of urban birds has recently taken place [56], coinciding with the increasing installations of cellsites. Although this type of contamination is considered at the present time by some experts as the most serious [4], inspection systems and controls have never been developed to avoid their pernicious effects on living organisms. Some of the biological mechanisms of the effects of these waves are still ignored [12], although the athermal effects on organisms have been sufficiently documented. The telephone industry could be taking advantage of the complexity of the biological and physical processes implied, to create an innocuous atmosphere, repeatedly denying the existence of harmful effects in living organisms. For this reason the reports related to animals are of special value, since in this case it can never be alleged that the effects are psychosomatic [3].

Future investigation should be carried out with long-term monitoring of the breeding success, of the sleeping places and of the uses of the habitat for species more vulnerable to the microwaves. Of special interest should be investigations that try to make correlations with the radiofrequency electromagnetic field measurements. Field studies investigating populations of urban parks and territories surrounding cellsites should be a high-priority. A radius of 1 sq K and the layout of concentric lines at intermediate distances can be useful to investigate differential results among areas depending on their vicinity and the radiation levels. We consider that the birds most affected from the microwave electromagnetic contamination could be:

- 1) those bound to urban environments with more sedentary customs, in general those that spend more time in the vicinity of the base stations;
- 2) those that live or breed in high places, more exposed to the radiation and at higher power density levels;
- 3) those that breed on open structures where the radiation impacts directly on adults and chicks in the nest;
- 4) those that spend the night outside of holes or structures that attenuate the radiation.

In far away areas, where the radiation decreases progressively, the chronic exposure can also have long term effects [13, 49]. Effects from antennas on the habitat of birds are difficult to quantify, but they can cause a serious deterioration, generating silent areas without male singers or reproductive couples. The deterioration of the ecosystem can also take place from the impact of the radiation on the populations of invertebrate prey [54, 57, 58] and on the plants [59].

Bioelectromagnetics is historically a frontier discipline. Controversy is frequent when the scientists recognize serious effects on health and on the environment that cause high economic losses. Independent investigators state the necessity of a drastic reduction of the emitted power levels on people and the ecosystems and that it is technically viable although more expensive for the industry [4, 22, 60]. Our opinion is that areas of continuous use should never exist at the height of the antennas either inside the beam or within a radius of several hundreds meters. The restriction to exposure to fauna presents special complexity; the main reason for the drastic reduction in the emission power of the antennae is presented as the only viable and effective solution to prevent these effects. Some authors have already propose that we are witnessing a paradigm change in biology [61].

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RF Radiation–Induced Changes in the Prenatal Development of Mice

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The possible effects of radiofrequency (RF) radiation on prenatal development has been investigated in mice. This study consisted of RF level measurements and in vivo experiments at several places around an “antenna park.” At these locations RF power densities between 168 nW/cm² and 1053 nW/cm² were measured. Twelve pairs of mice, divided in two groups, were placed in locations of different power densities and were repeatedly mated five times. One hundred eighteen newborns were collected. They were measured, weighed, and examined macro- and microscopically. A progressive decrease in the number of newborns per dam was observed, which ended in irreversible infertility. The prenatal development of the newborns, however, evaluated by the crown-rump length, the body weight, and the number of the lumbar, sacral, and coccygeal vertebrae, was improved. *Bioelectromagnetics* 18:455–461, 1997. © 1997 Wiley-Liss, Inc.

Key words: RF radiation effects; prenatal development; mice development

Five years ago the “antenna-park of Thessaloniki” progressively developed on the top of the nearby mountain Chortiatis, 1.5 km away from a small village of the same name. Today, almost 100 commercial TV and FM-radio broadcasting transmitters in the VHF and the UHF bands are situated there. The antennas are installed on towers well visible from a large part of the village. Living so close to the antennae and the vast amount of RF power they transmit, which is of the order of 300 kW, the people of the village Chortiatis, anxious for their health, encouraged the author to undertake a research program.

The hypothesis that RF radiation may adversely affect the health of the animal organism is still under consideration in public and scientific forums. One of the critical issues seems to be the RF effects on the reproductive process [Chernoff et al., 1992]. Numerous studies dealing with this subject ended up with seemingly contradictory results. Therefore, an “in vivo” study on experimental animals sensitive to RF radiation, was chosen. Based on the relevant literature, this research investigated RF radiation effects on the reproductive system, particularly on prenatal development. The mouse was selected as the experimental animal, because it is easily manipulated in the environment in which the experiments had to take place. Of course, experimenting at the mountain sites, far from the easily

controlled laboratory conditions, might add a certain amount of uncertainty; therefore, these experiments should be considered preliminary.

MATERIALS AND METHODS

We used a total of 36 mice (18 females and 18 males), 2 months old and sexually mature (BALB/c/f breed colony). Breeding colony virgin males and females were obtained from the “Theageneion Anticancer Institute of Thessaloniki.” The use of these experimental animals was approved by the Veterinary Service of the Municipality of Thessaloniki, according to the provisions of the laws 1197/81 and 2015/92 and the Presidential Decree 160/91 of the Greek Democracy. Upon arrival, all experimental animals were quarantined for 2 weeks to discover and to allow them to acclimatise the mountain environment, an altitude ranging between 570 (position h) and 730 m (position d) above sea level. All the mice were healthy at the end of this period and showed no signs of illness during

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TABLE 1. Light–Dark Cycle during the Experimental Matings

Gestation	Date	Day		Night	
		Min	Max	Min	Max
1 st	25.5–16.6	14.28	14.47	09.13	09.32
2 nd	21.6–12.7	14.37	14.48	09.12	09.23
3 rd	6.9–29.9	11.54	12.45	11.15	12.06
4 th	7.10–28.10	10.45	11.35	12.25	13.15
5 th	23.11–13.12	09.34	09.55	14.05	14.26

the course of the study. Tap water and certified feed (Greek Sugar Factory) were freely available.

The mice were maintained under natural lighting, both during the daytime and at the night (Table 1). Twelve Plexiglas cages transparent to RF radiation, were placed at several locations with one female in each cage. Each female was caged with one male for 12 h. Vaginal smears were taken the next morning and successful mating was identified by the presence of sperm. The day on which evidence of mating was observed was considered to be the first day of gestation. The litters were collected in the first 2 h after delivery and were moved to the laboratory for examination. After a period of recovery, the same mating procedure was repeated for each dam. Five experimental pregnancies were carried out in a period of almost 6 months.

The first pregnancy of the experimental animals took place in eight selected positions (a–h, Fig. 1), some close to the “antenna-park” and some near the village of Chortiatis. Then the experimental animals were moved to two positions, because these positions presented almost the same RF radiation levels with those initially selected and the experiment could be managed more effectively. Six dams (labelled as group A), initially placed at positions a, b, c, and d, with their males, were moved to the position d (Refuge of Hypaithrios Life). The other six dams (labelled group B), with their males, initially placed at positions e, f, g, and h were moved to position h (Public Primary School of Chortiatis). These two positions were selected because the most important living conditions, i.e., light, temperature, ventilation, food, etc., were the same.

Finally, all the experimental animals were moved to position i (Laboratory of Anatomy, School of Veterinary Medicine, University of Thessaloniki) about 10 km away from the Mountain Chortiatis, in the city of Thessaloniki, for the fifth pregnancy. This relocation was done to seek an indication of a possible reversibility of the observed phenomena. In fact, we wanted to repeat the experiment in an environment almost free of RF. An extra group of six couples of mice were mated once and used as controls in the laboratory (posi-

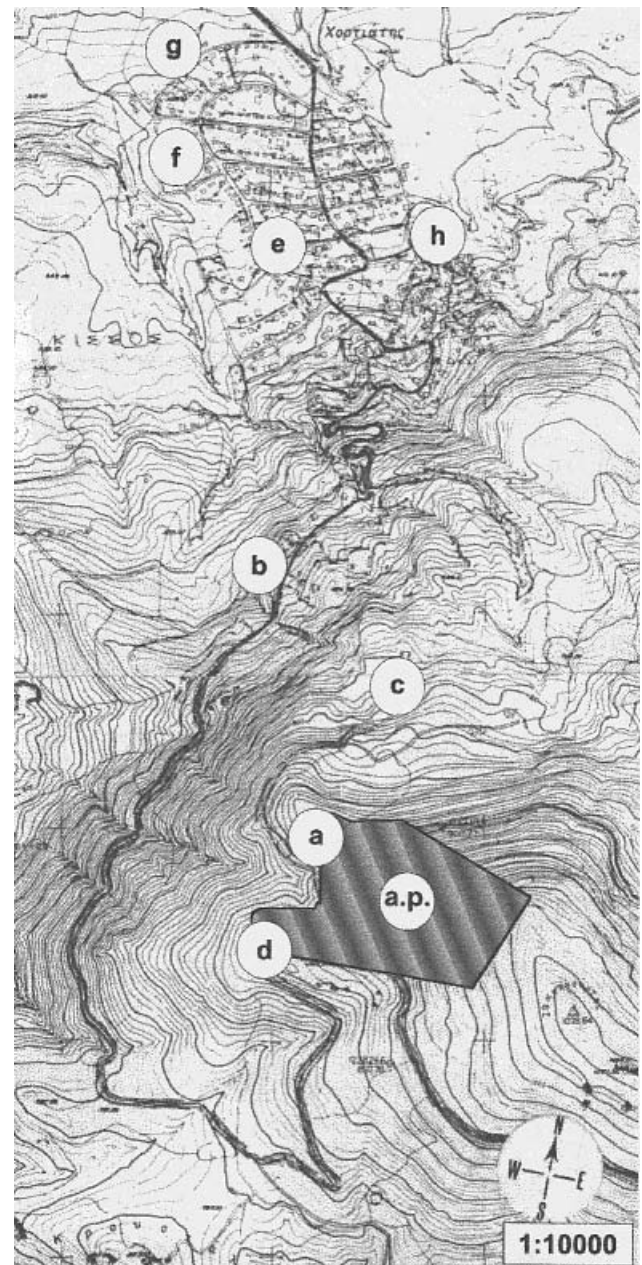


Fig. 1. Wide area of Chortiatis, where the first four matings took place.

tion i), far from the "antenna park" in a more or less free-of-RF radiation environment.

It was extremely difficult to use RF-free controls at the mountain sites, because it was almost impossible to make "electromagnetically screened cages." Such a cage should ideally provide high (of the order of 30 dB) screening at the frequency range between 88.5 and 950 MHz (Commercial Radio FM band, UHF TV band, and Mobile Communication band), and therefore would require a very dense and well-grounded, highly conductive external metal grid. Obviously, mice could hardly survive in such cages for about 5 months.

The litter was considered to be the experimental unit for the analysis of data. We measured the crown-rump length, the body weight, the number of the posterior (lumbar, sacral, and coccygeal) vertebrae, the congenital malformations, and the ossification of the skeleton.

The RF power was measured in each position, using an electric field meter and a low gain (4 dB) wide-band (80–900 MHz) log-periodic antenna and spectrum analyser. To obtain comparable results the "IEEE std. C95.3.1991" was used. On the third floor of the public school, where the mice were situated, a 360 degree integration was also performed, due to the directivity of the measuring antenna together with the close proximity of the walls and metal furniture. Whenever iron bars or metal screens existed in front of the windows, two series of measurements were carried out; one on each side of the screen.

The collected newborns were killed for examination. Their crown-rump length was measured, and they were weighed and inspected under the dissecting microscope for external congenital malformations. Then they were fixed and subsequently cleared and stained in toto by a double staining of their skeleton [Peters, 1977]. The procedure was lightly modified as follows:

The newborns were fixed with alcohol 86% for 3 days; their skin, eyes, and viscera were removed; then they were immersed for 3 days in alcohol 100% and for 4 days in a mixture of alcohol 100% and ether 1:1. They were stained for 1–2 days with blue alcyan coloration [alcohol 86% 80 ml, acetic acid 20 ml, alcyan blue 20 mg] until the nonmineralised cartilaginous parts of the bones became blue. They were immersed in alcohol 100% for 4 days. Then they were stained for 12–24 days with red alizarin coloration [KOH 1 g, H₂O 100 ml, alizarin solution (alcohol 86% saturated with alizarin red S) 0.1 ml] until the ossified parts of the bones became red. They were immersed in solution Mall I (KOH 1 g, distilled water 80 ml, glycerine 20 ml) until the transparency of their body was completed. Finally, they were stored in a conservation solution (distilled water and glycerine 1:1, with

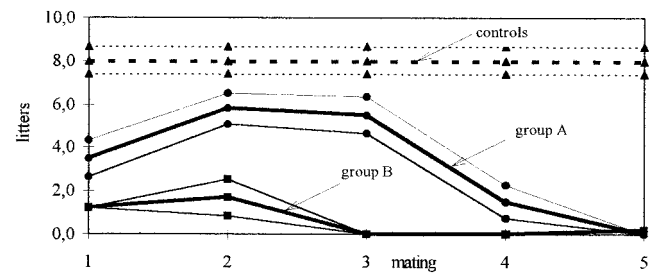


Fig. 2. Comparison of the mean values \pm standard deviation of number of newborns per dam and mating from all experimental groups.

some thymol crystals as contamination prevention). The stained newborns were inspected for skeletal defects as well as for the degree of ossification of their bones. The ossification of the skeleton and particularly of the vertebrae is an excellent and creditable indicator of the prenatal exposure to noxious agents and can be a measure of development delay.

RESULTS

The RF power levels measured, although below the limits proposed by the "ENV50166-2" and the "IEEE C95.1.1991" standards, are high and well above the power levels that are likely to be measured in other European or U.S. residential areas. In fact, on the third floor of the public primary school (position h), an average power density of $1.053 \mu\text{W}/\text{cm}^2$ was found, equivalent to a specific absorption rate of $1.935 \text{ mW}/\text{kg}$. In the Hypaithrios Life Refuge (position d) the average power density in which the mice were located was of the order of $168 \text{ nW}/\text{cm}^2$. This reduced level was due to the screening effect of the iron bars in front of the windows, which gave an 8–10 dB RF-power decrease. The average power density levels in position i (Laboratory of Anatomy, School of Veterinary Medicine, University of Thessaloniki), where the controls were placed and the fifth experimental matings were performed, was 40 dB weaker.

The number of the littered newborns by the experimental dams of groups A and B were, compared with those littered by the controls, progressively reduced from the first to the fifth pregnancy. This reduction is more evident in group B and is clearly shown in Table 2 and in Figure 2.

On the other hand, the rest of the four measured parameters, i.e., the crown rump length and the weight and the number of the lumbar, sacral, and coccygeal vertebrae increased in the newborns from groups A and B compared with the controls. This was more evident in group A than in group B (Table 2 and Fig. 3). A

TABLE 2. Statistical Characteristics of All Four Measurable Parameters per Dam, per Group, and per Gestation

Mating	Litters per dam mean \pm s.d. median	Length (cm)	Weight (gr)	Vertebrae
Group A (6 dams)				
1 st (25.05.1995)	3.5 \pm 0.9 4.0	1.47 \pm 0.13 1.44	2.71 \pm 0.09 2.69	31.48 \pm 1.43 32.07
2 nd (21.06.1995)	5.8 \pm 0.7 7.0	1.25 \pm 0.06 1.22	2.55 \pm 0.05 2.50	24.28 \pm 0.97 24.29
3 rd (08.09.1995)	5.5 \pm 0.9 6.5	1.72 \pm 0.25 1.72	2.71 \pm 0.13 2.60	28.72 \pm 1.92 28.71
4 th (07.10.1995) ^a	1.5 0.0	1.10 1.10	2.47 2.47	23.22 23.22
5 th (23.11.1995) ^a	0.0 0.0			
Mean value	3.3	1.39	2.61	26.93
Group B (6 dams)				
1 st (25.05.1995) ^a	1.2 0.0	1.19 1.19	2.53 2.53	28.57 28.57
2 nd (21.06.1995)	1.7 \pm 0.9 1.5	1.25 \pm 0.04 1.26	2.60 \pm 0.06 2.58	28.55 \pm 1.14 27.26
3 rd (08.09.1995) ^a	0.0 0.0			
4 th (07.10.1995) ^a	0.0 0.0			
5 th (23.11.1995)	0.2 0.0	1.05 1.05	2.50 2.50	30.00 30.00
Mean value	0.6	1.16	2.54	29.04
Controls (6 dams)				
1 st (23.11.1995)	8.0 \pm 0.07 7.5	0.96 \pm 0.15 0.97	2.38 \pm 0.02 2.37	19.59 \pm 0.47 19.52
Mean value	8.0	0.96	2.38	19.59

^aSingle or no gestation.

thorough external and internal examination under the dissecting microscope revealed only one case of extensive and two cases of limited malformation. No retarda-

tion of skeletal ossification worth mentioning was observed; only five cases out of 116 showed limited retardation. It has to be noted here, that the evaluation of the skeleton ossification was focused in the bones of the forelimbs and hindlimbs and in the lumbar, sacral, and coccygeal vertebrae.

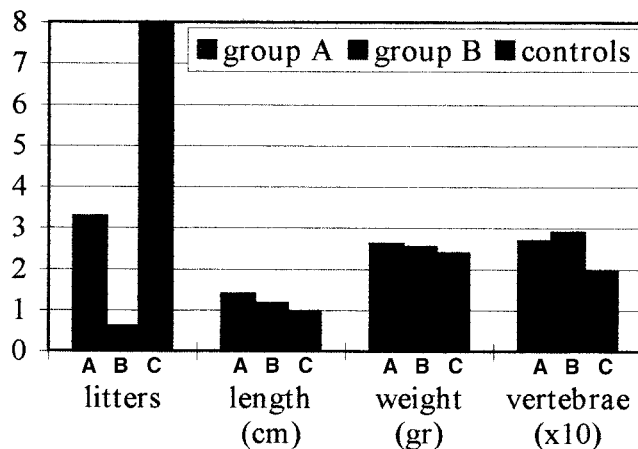


Fig. 3. Comparison of the mean values of all four measurable parameters for all gestations. Controls (C).

DISCUSSION

To study effects of a possibly noxious agent on a mammalian embryo, three groups should be considered: the embryos, the dams, and the males. In this work, all three have been studied: the infertility for dams and males, the lethality for embryos, the teratogenicity or the reduction in deformity for foetuses, or any combinations of them. They all have been considered by exposing male and female mice (before and during pregnancy) to an RF-radiation environment close to the "antenna park."

Infertility and lethality were assessed by counting the number of their newborns, whereas the possible

teratogenicity and the reduction deformity by autopsy was considered by the study of the embryonic skeletons. An important stage in this study was the examination of the skeletons, since the ossification of the bones is considered an excellent and creditable indicator of the prenatal exposure to noxious agents and can be a measure of development delay. In the beginning of organogenesis, the neural tube functions as a precursor of the cartilages and bones of the developing skeleton [Noden and Delahunta, 1985]. Teratogenic factors of any kind, that affect the embryonic nervous system, result in structural defects of the skeletal components. Therefore, to detect the teratogenic action of a factor on the embryonic nervous system, it is technically convenient to study the foetal skeleton rather than the embryonic nervous system itself.

A very important result of this experimental study (Table 2 and Fig. 2) is a progressive decrease of the number of the size of the litters of the dams of group A (position d) and group B (position h), compared with the controls (position i) and with the breeding history of these mice. Mice from the BALB/c/f breeding colony obtained from the "Theageneion Anticancer Institute of Thessaloniki" have been used for years in our laboratory for reproduction. Repeated pregnancies with a recovery period of 1–4 weeks for over a year, had never affected the fertility of the dams or any morphological parameters of the offspring, a fact that to our knowledge has not been questioned in the available literature.

It is worth noting that the RF power density levels, although very different from place to place, were very low and well below the CENELEC and IEEE relevant standards. Yet, it should be pointed out that:

(a) the experimental animals lived in this environment for 6 months, which is a long period of time,

(b) there was a considerable difference in power density levels of the order of 10 dB between the two main positions d and h and almost of 40 dB between d and i,

(c) there is a considerable difference between the volumes and consequently the body mass of the adult mouse and other experimental animals used as models in the international standards applied to humans.

The interpretation of our observations could follow various directions. The most popular view in numerous studies of the relevant literature, that this is a consequence of the overheating of the irradiated testis [Lary et al., 1986, 1987; O'Connor, 1980] could be considered. On the other hand, the assumption that RF and microwave radiation effects are limited to heating has been questioned in a series of studies [Cleary, 1988, 1990]. The exposure conditions in these "in vivo" studies may suggest a thermal component of RF-in-

duced testicular damage. However, interpretation of these data with respect to damage thresholds or interaction mechanisms is difficult. This difficulty is due to a number of factors, including the time, intensity, or both, the variations in species sensitivities, and the frequency-dependent non-uniform microwave energy absorption in tissue. Consequently, although these findings seem to be consistent with a hypothesis that the RF-induced heating is associated with testicular damages, the borderline between the "direct" effects of radiation and the effects that are indirectly associated with the tissue heating is not very clear.

Our observations could also be attributed to an intra-uterus death of the irradiated embryos in the early stages of the prenatal development, a speculation that could not be investigated in our experimental design because it required a postmortem autopsy of the dam. On the other hand, the prerequisite to these scenarios is a large RF power density, whereas the power densities we measured were of the order of $\mu\text{W}/\text{cm}^2$ or nW/cm^2 , rather than mW/cm^2 , or in terms of specific absorption rate (SAR), mW/kg rather than W/kg . Therefore, we cannot exclude the possibility of an indirect nonthermal mechanism focused on the endocrinological axon hypophysis-gonads that causes infertility to the males or the females [Thuery, 1991].

It should be noted here that the male experimental animals progressively developed a very bad physiological condition (rough hair, emaciation, etc.), not correlated to any other sickness symptoms, during their stay at the experimental positions a–g. Therefore, despite of the limited amount of data, the duration of the exposure to low intensity RF electromagnetic fields seems to be a repression parameter. In fact, chronic or long-term exposure to low intensity electromagnetic fields is generally associated with adverse results [Lary et al., 1983]. The most peculiar findings of this study were the increases in the crown-rump length, the body weight, and the number of the posterior vertebrae (lumbar, sacral, and coccygeal) of the experimental offsprings compared with the controls (Table 2, Fig. 3).

It must be noted that a study of mice [Jensh et al., 1977; 1978a; 1978b] under low levels of irradiation during the whole period of a single gestation (10 and 20 mW/cm^2) had no effect on maternal, foetal, or placental masses and no effect on the frequency of resorption, foetal death rate, size of litter, sex of the newly born, and their ability to perform. Other studies [Michaelson et al., 1976] reported a faster development of rat foetuses. This finding agrees with another report [Johnson et al., 1977] that noted an increase in the weight of newly born rats and a premature opening of the eyes after prenatal irradiation (5 mW/cm^2 at 918 MHz, for 380 h), as well as an impaired ability to learn. On the

other hand, other studies found lower average weight at birth. At medium power density levels (10, 20, and 50 mW/cm², at 2375 MHz), which are above the limits imposed by CENELEC and the relevant IEEE standard, the reproductive capacity of mice was somewhat impaired, with smaller litter size and a rise in neonatal mortality, which is a direct function of the power flux density [Il'cevic and Gordodeckaja, 1976; McRee, 1980].

Although it is difficult to explain this foetal development increase, we believe that it could be due to a favourable placental nourishment of the foetuses during the pregnancy. In fact, this finding could be associated with:

(a) reproductive causes, i.e., blood-flow to a smaller number of foetuses, because of the reduction of the fertility of the irradiated males or females,

(b) thermal causes, i.e., possible increase of the blood flow of the dams, directly due to the RF irradiation,

(c) endocrinological causes, i.e., increase of the somatotrophic hormone because of the RF irradiation and

(d) environmental causes, i.e., the vasodilatation and partial increase of the blood pressure of the experimental dams because of the mountain altitude.

Of course combinations of these possibilities cannot be excluded.

According to various references [Tell and Harlen, 1979; Lu et al., 1980; Deschaux et al., 1983] discrepancies between the results of experiments may be due to different experimental conditions, random formation of hot spots in the glands and the hypothalamus, or a variety of other factors, as the circadian rhythm and differences between species. With the exception of the high power effects on testicles, that do not belong to the endocrine ensemble, the interaction seems to involve the pituitary gland or even the central nervous system rather than the terminal glands.

We would close this discussion with what Jacques Thuery wrote (1991), that the true state of affairs is probably far more complex, but the available data are not sufficient to allow us to outline it more clearly, and that all attempts to extrapolate these results to humans lead to very high power densities, partly because geometric resonance effects are very significant in small animals. Consequently, taking into account the constant exposure of the human population living close to the "antenna park" to low intensity RF radiation, these adverse health effects in mice resulting from chronic or prolonged exposure may prove of importance in the near future. Indeed, there is evidence that chronic exposure to low-intensity RF radiation may be associ-

ated with health effects different to embryo-toxicity [Salford et al., 1992; Cleary, in press].

The findings of this preliminary experimental study have led to several conclusions. Of course, the final word to the problem in question has not been said as yet. Therefore, more work is called for; laboratory-based simulation might provide valuable information.

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